

AMENDMENT TO THE CLAIMS

Applicants respectfully request that claims be amended without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents. The following listing of claims will replace all prior versions and listings of claims in the application.

1. (Currently amended) A method for preparation of purifying Interleukin-4 (IL-4) or muteins thereof of Interleukin 4 by recombinant expression comprising: (a) expression expressing the IL-4 or muteins thereof in a prokaryotic cell thereby forming in inclusion bodies containing IL-4 or muteins thereof in said prokaryotic cell; (b) disrupting the cells prokaryotic cell to release the inclusion bodies; and (c) separating the inclusion bodies from the cell debris; (e) washing inclusion bodies so obtained; (d) solubilizing the inclusion bodies in a solution that includes a denaturing agent, thereby denaturing the IL-4 or muteins thereof by denaturation; (e) separating the denatured IL-4 or muteins thereof using an immobilized metal chelate affinity chromatography (IMAC) system; and (f) renaturating (f) releasing the IL-4 or muteins thereof from the IMAC system, thereby obtaining a renatured IL-4 or muteins thereof expression product and (f) purifying the expression product characterized in that the inclusion bodies are washed (e) with a buffer containing a detergent which effectively solubilizes lipids bound to the surface of the inclusion body or lipids contained in cell wall fragments.

2. (Currently amended) The method according to claim 1, wherein the step of separating the inclusion bodies further comprises the step of washing the inclusion bodies in a washing

buffer detergent capable of solubilizing lipids bound to the surface of the inclusion bodies or contained in cell wall fragments for washing the inclusion bodies is a non-ionic detergent, a ionic surfactant or a zwitterionic detergent.

3. (Currently amended) The method according to claim 42, wherein the detergent washing buffer is a non-ionic detergent, an ionic surfactant or a zwitterionic detergent. ~~selected from the group CHAPS, CHAPSO, desoxycholate and the zwittergent series (N-alkyl N,N-ditnethyl-3-ammonio-1-propanesulfonate)~~.

4. (Currently amended) The method according to claim 42, wherein the washing buffer for the inclusion bodies is a buffer which maintains the pH between 7 and 10.

5. (Currently amended) The method according to claim 42, wherein the washing buffer additionally contains a chelating substance.

6. (Currently amended) The method according to claim 5, wherein the chelating substance is selected from the group consisting of ethylenediamintetraacetic acid (EDTA), ethyleneglycol-O,O'-bis-(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), nitriloacetic acid (NTA) or and trans-1,2-diamino-cyclohexan-N,N,N',N'-tetraacetic acid (CDTA).

7. (Currently amended) The method according to claim 1, wherein the recombinant protein is Interleukin-4 is IL-4 R121D Y124D.

8. (Currently amended) The method according to claim 1, wherein the ~~renaturation (e)~~
renatured IL-4 or muteins thereof are is done obtained by dialysis, diafiltration or dilution
optionally in the presence of artificial chaperones.

9. (Currently amended) The method according to claim 8, wherein the ~~renaturation (e)~~
dialysis, diafiltration or dilution is done in the presence of an artificial chaperones.

10. (Currently amended) The method according to claim 1, wherein ~~the purification (f) is~~
~~done by chromatography~~ the artificial chaperone is a cyclic dextrin or linear dextrin.

11. (New) The method according to claim 1, wherein the prokaryotic host is *E. coli*.

12. (New) The method according to claim 1, wherein the IL-4 is mIL-4 Q116D Y119D.

13. (New) The method according to claim 1, wherein the step of separating the inclusion
bodies is carried out by centrifugation.

14. (New) The method according to claim 1, wherein the solution used in the step of
solubilizing the inclusion bodies comprises guanidinium salts.

15. (New) The method according to claim 1, further comprising a step of renaturing the

denatured IL-4 or muteins thereof prior to step (f).

16. (New) The method according to claim 15, wherein the renaturing step is by matrix-assisted refolding wherein the IL-4 remains bound to the IMAC system.

17. (New) The method according to claim 1, wherein the step of separating the denatured IL-4 or muteins thereof with the IMAC system provides the IL-4 or muteins thereof having a purity of about 90% as estimated by SDS-PAGE analysis.

18. (New) A pharmaceutical composition comprising a purified IL-4 or a mutein thereof obtained by the method of claim 1 and a pharmaceutically acceptable carrier.

19. (New) The method according to claim 3, wherein the zwitterionic detergent is selected from the group consisting of CHAPS, CHAPSO, desoxycholate and the zwittergent series (N-alkyl-N,N-ditnethyl-3-ammonio-1-propanesulfonate).